## CLAIMS

What is claimed is:

1.  A method for detecting in real time the amount of a target nucleic acid molecule in a sample, wherein the melting of the target nucleic acid molecule starts at a temperature $T_{MS}$ and completes at a temperature $T_{ME}$, the method comprising:

> A.  Establishing a standard curve by:

>> i) PCR-amplifying, in the presence of a suitable fluorescent dye, the target nucleic acid molecule, with a known starting concentration (C) through cycles of denaturing, annealing, and chain extension, wherein the fluorescence is increased when the dye is combined with a double-stranded nucleic acid molecule, wherein the chain extension occurs at a chain extension temperature $T_E$;

>> ii) measuring the fluorescence (F) during each amplification cycle at the temperature immediately before the temperature starts to increase from $T_E$ ($F_E$ at $T_E$), at any temperature point ($T_B$) in between $T_E$ and $T_{MS}$ ($F_B$ at $T_B$), at $T_{MS}$ ($F_{MS}$ at $T_{MS}$ )and at $T_{ME}$ ($F_{ME}$ at $T_{ME}$);

>> iii) calculating a baseline slope ($S_B$), defined by negative ($F_B$ minus $F_E$), divided by ($T_B$ minus $T_E$), and an amplicon melting phase slope ($S_M$), defined by negative ($F_{ME}$ minus $F_{MS}$) divided by ($T_{ME}$ minus $T_{MS}$);

>> iv) recording the number of PCR cycles (N) required for the quantity ($S_M$ minus $S_B$) to first become greater than zero;

>> v) repeating steps i) through v) for a suitable range of concentrations of interest; and

>> vi) plotting C against $C_T$ to obtain a standard curve for the target nucleic acid sequence; and

> B.  Repeating steps (A) (i) through (A)(v) for a sample containing an unknown concentration of the target nucleic acid molecule, to obtain an $C_T$ value for the sample, and determining the target nucleic acid molecule concentration via the standard curve.

2.  The method of Claim 1 wherein the sample contains a first target nucleic acid molecule and a second target nucleic acid molecule, wherein the melting of the first target nucleic acid molecule starts at a temperature $T_{MS1}$ and completes at a temperature $T_{ME1}$, the melting of the second target nucleic acid molecule starts at a temperature $T_{MS2}$ and completes at a temperature $T_{ME2}$, and wherein $T_{MS2}$ is greater than $T_{ME1}$, the method comprising:

> A.  Establishing a standard curve for each of the target nucleic acid molecule by:

>> i) simultaneously PCR-amplifying, in the presence of a suitable fluorescent dye, the target nucleic acid molecules with a known starting concentration ($C_1$ and $C_2$) through cycles of denaturing, annealing, and chain extension, wherein the fluorescence is increased when the dye is combined with a

double-stranded nucleic acid molecule, wherein the chain extension occurs at a chain extension temperature $T_E$;

    ii) measuring the fluorescence (F) during each amplification cycle at the temperature immediately before the temperature starts to increase from $T_E$ ($F_E$ at $T_E$), at any temperature point ($T_{B1}$) in between $T_E$ and $T_{MS1}$ ($F_{B1}$ at $T_{B1}$), at $T_{MS1}$ ($F_{MS1}$ at $T_{MS1}$), at $T_{ME1}$ ($F_{ME1}$ at $T_{ME1}$), at any time point ($T_{B2}$) in between $T_{ME1}$ and $T_{MS2}$ ($F_{B2}$ at $T_{B2}$), at $T_{MS2}$ ($F_{MS2}$ at $T_{MS2}$), at $T_{ME2}$ ($F_{ME2}$ at $T_{ME2}$);

    iii) calculating a baseline slope for the first target molecule ($S_{B1}$), defined by negative ($F_{B1}$ minus $F_E$), divided by ($T_{B1}$ minus $T_E$), and a first amplicon melting phase slope for the first molecule ($S_{M1}$), defined by negative ($F_{ME1}$ minus $F_{MS1}$) divided by ($T_{ME1}$ minus $T_{MS1}$); and calculating a baseline slope for the second target molecule ($S_{B2}$), defined by negative ($F_{B2}$ minus $F_{ME1}$), divided by ($T_{B2}$ minus $T_{ME1}$), and a melting phase slope for the first molecule ($S_{M2}$), defined by negative ($F_{ME2}$ minus $F_{MS2}$) divided by ($T_{ME2}$ minus $T_{MS2}$);

    iv) recording the number of PCR cycles ($N_1$) required for the quantity ($S_{M1}$ minus $S_{B1}$) to first become greater than zero; and recording the number of PCR cycles ($N_2$) required for the quantity ($S_{M2}$ minus $S_{B2}$) to first become greater than zero;

    v) repeating steps i) through v) for a suitable range of concentrations of interest for each of the two target molecules; and

    vi) plotting $C_1$ against $N_1$ to obtain a standard curve for the first target molecule; and plotting $C_2$ against $N_2$ to obtain a standard curve for the second target molecule; and

B. Repeating steps (A) (i) through (A)(v) for a sample containing an unknown concentration of the first and second target nucleic acid molecules, to obtain an $N_1$ value and an $N_2$ value for the sample, and determining the target nucleic acid molecule concentrations via the standard curve.

3. The method of Claim 1 wherein the sample contains a first target nucleic acid molecule, a second target nucleic acid molecule and a third target nucleic acid molecule, wherein the melting of the first target nucleic acid molecule starts at a temperature $T_{MS1}$ and completes at a temperature $T_{ME1}$, the melting of the second target nucleic acid molecule starts at a temperature $T_{MS2}$ and completes at a temperature $T_{ME2}$, the melting of the third target nucleic acid molecule starts at a temperature $T_{MS3}$ and completes at a temperature $T_{ME3}$, and wherein $T_{MS3}$ is greater than $T_{ME2}$, the method comprising:

A. Establishing a standard curve for each of the target nucleic acid molecule according to the method of Claim 1;

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B. Simultaneously PCR amplifying a sample containing an unknown concentration of the target nucleic acid molecules, to obtain an $N_1$, $N_2$ and $N_3$ value for the sample, and determining the target nucleic acid molecule concentrations via the standard curve.

4. The method of Claim 1 wherein the sample contains n target nucleic acid molecules, wherein n is an integer greater than three, wherein the melting of the first target nucleic acid molecules starts at a temperature $T_{MS1}$ and completes at a temperature $T_{ME1}$, the melting of the second target nucleic acid molecule starts at a temperature $T_{MS2}$ and completes at a temperature $T_{ME2}$, the melting of the (n-1)$^{th}$ target nucleic acid molecule starts at a temperature $T_{MS(n-1)}$ and completes at a temperature $T_{ME(n-1)}$, the melting of the n$^{th}$ target nucleic acid molecule starts at a temperature $T_{MSn}$ and completes at a temperature $T_{MEn}$, and wherein $T_{MSn}$ is greater than $T_{ME(n-1)}$, the method comprising:

A. Establishing a standard curve for each of the target nucleic acid molecule according to the method of Claim 1;

B. Simultaneously PCR amplifying a sample containing an unknown concentration of the target nucleic acid molecules, to obtain an $N_1$, $N_2$ . . . and $N_n$ value for the sample, and determining the target nucleic acid molecule concentrations via the standard curve.

5. The method of Claim 2, wherein the first and the second target nucleic acid molecules reside on the same genome of an organisms, and wherein the copy number per genome for the first target nucleic acid molecule is known, whereby the copy number per genome for the second target nucleic acid molecule is determined.

6. The method of claim 1, wherein the target nucleic acid molecule is from a pathogenic organisms.

7. The method of Claim 2, wherein the first target nucleic acid is an invertase gene, an aldolase gene or a lectin gene, and wherein the second target nucleic acid is selected from the group consisting of the 35S CaMV promoter, a Cry9C gene, an GA21 gene, an EPSPS (5-enolpyruvylshikimate-3-phosphate synthase gene, a PEPC promoter; an hsp70 promoter of Cry1A(b) gene, a Cry1A(b) gene; an NOS gene, and the actin promoter gene.

8. The method of Claim 1 wherein the target nucleic acid molecule is selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:9, and SEQ ID NO:10.

9. The method of claim 1, wherein the target nucleic acid molecule is a nucleic acid fragment is part of a transgene contained in a genetically modified organism.
The method of claim 9 wherein the target nucleic molecule comprises a promoter for the transgene.

10. The method of claim 10 wherein the promoter is the 35S promoter of Cauliflower Mosaic Virus.

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